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Effects of Extraction Time on the Functional Properties of Silver Catfish *(Pangasius sutchi)* Skin Gelatin

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ABSTRACT

Silver catfish (Pangasius sutchi) skin gelatin was extracted to determine the effects of extraction time on the functional properties of the gelatin in terms of solubility, protein solubility as a function of pH and sodium chloride concentration, emulsifying capacity and stability, water holding capacity, fat binding capacities and foaming properties. Silver catfish skins were washed in sodium chloride (NaCl) solution prior to pre-treatment in sodium hydroxide (NaOH) and acetic acid solution. Gelatin was extracted at 50ºC for 6, 8, 10 and 12 hours extraction time followed by freeze drying. The extraction of silver catfish skin gelatin at 50 ºC for 12 hours was more effective than extraction at 6, 8 and 10 hours where the gelatin was characterized by higher emulsifying capacity (52.63%), emulsifying stability (47.83%), water holding capacity (31.78 mL/g), fat binding capacities (54.76%), foaming capacity (41.47 mL) and foaming stability (56.42%) than gelatins extracted at other extraction time. The longer the extraction time, the better the functional properties of the gelatin. Based on its good functional properties, silver catfish skin gelatin may be useful in various food applications such as soups, sauces and gravies.

Keywords: *silver catfish (Pangasius sutchi), gelatin, extraction time, functional properties, emulsifying, foaming*

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Introduction

Freshwater fish has become a significant fish resource in Malaysia. According to the Department of Fisheries Malaysia [1], 29.1% of the total aquaculture production was contributed by freshwater aquaculture, increased from 61,652.48 tons to 70,064.27 tons by 12% in 2007. The major freshwater species cultures were red tilapia (26,175.33 tons), catfish (21,891.55 tons), black tilapia (5,848.98 tons) and *Pangasius* catfish (5,784.44 tons). *Pangasius sutchi* also known as *sutchi* catfish, iridescent shark-catfish, striped catfish, 'Pa sooai' and 'Pa sooai khaeo' in Laotian, 'Pla Sawai' in Thai, 'Pra' and 'Trey pra' in Khmer and 'Cá Tra' in Vietnamese is popular among consumers worldwide [2-3]. In some countries, silver catfish is mostly marketed in fresh seafood market and supermarkets as frozen or thawed fillets [4].

Skin and bone are commonly used as raw materials for gelatin extraction [5]. Gelatin is usually extracted from mammalian sources [6]. The annual world output of gelatin was nearly 326,000 tons, with the highest (46%) derived from pig-skin followed by bovine hides (29.4%), bones (23.1%) and other sources (1.5%) [7]. Mammalian gelatin has been widely utilized due to its gelling point, high melting and thermo-reversibility, high in molecular weight and water-soluble protein [8 - 9]. However, due to outbreak of bovine spongiform encephalopathy (BSE) as well as religious and social reasons, there has been an interest in gelatin production from fish sources [10].

The surimi and fillet processing industry are fish-based industry in Malaysia and is developing progressively due to high demands for fish-based products in the market [7]. Processing leads to the generation of large amount of waste such as skin, bones, and fins which are generally discarded (about 7.3 million tons/year) [11]. Freshwater fish skin that comprises about 5% of the whole fish has become an interesting raw material for producing gelatin [7]. Fish skin is especially suitable as a source of gelatin because it is easily extracted with high yield at relatively moderate temperature, usually at or below 50°C [12].

Gelatin extraction from the skin of silver catfish could be an effective method to reduce the wastage of fish skin and pollution problem. Besides, gelatin extracted from fish skin can be used as natural emulsifiers in foods since there are growing trend within the food industry to replace synthetic emulsifiers [13]. Extraction conditions such as temperature, pH and time

applied during both pre-treatment and extraction process affect the degree of conversion of collagen into gelatin which subsequently influence the length of polypeptide chains, functional properties, gelation of the gelatin and the type of gelatin produced [14-18]. In general, studies on the emulsifying and foaming properties of fish gelatin are very limited compared to studies on its gelation properties [3]. Thus, the study on the effects of different extraction time of silver catfish skin gelatin could solve the problem in terms of functionality in food applications. This study was carried out to analyze the effects of extraction time on the functional properties of silver catfish skin gelatin.

Materials and Method

Chemicals and Materials

All chemicals used are of analytical grade. Silver catfish (*Pangasius sutchi*) with the average weight of 800 g to 1000 g per fish and age around 6 months were obtained from a local freshwater fish breeder in Selayang, Selangor Malaysia.

Pre-treatment of Silver Catfish Skin

Silver catfish skin was pre-treated according to See *et al.* [7]. The skin was manually removed, cut into 2 to 3 cm pieces and washed in cold water (~5°C). Subsequently, the skin was washed with 0.8 M sodium chloride (NaCl) at the ratio of 1:6 (w/v) skin to NaCl solutions for two minutes followed by rinsing under running tap water.

Extraction of Gelatin

Extraction of gelatin was performed according to the method described by Tabarestani *et al.* [19]. The skin was initially soaked in 0.19 N of cold sodium hydroxide (NaOH) (~7°C) at the ratio of 1:3 w/v for 40 minutes to remove the non-collageneous protein. This was followed by rinsing using tap water and soaking in 0.12 N cold acetic acid solution (~7°C) at the ratio of 1:3 w/v for 40 minutes. The skin was then rinsed in distilled water until basic pH (pH 7) was achieved. Gelatin was extracted with distilled water at 50°C for 6, 8, 10 and 12 hours in a shaking water bath (Water Bath Shaking-1086,

Germany) followed by filtration through Whatman filter paper No. 42. The filtrate was freeze-dried and finally ground into a powder.

Functional Properties of Gelatin

Solubility of Gelatin

The solubility of gelatin was measured according to the method of Al-Kahtani and Hassan [20] with slight modification. 0.5 g gelatin powder (W_1) was placed in a glass beaker and added with 10 ml distilled water at 40 ºC. The powder was gently mixed with spatula until no more fine particles was visible. The solution was then filtered through a pre-weighed Whatman filter paper No.4 (W_2). The filter paper was dried at 100 ºC for 4 hours in a hot air oven (Memmert UNB 500, Germany), cooled in a desiccator and weighed again (W_3). Gelatin solubility was calculated as follows:

$$\% \text{ Solubility} = 100 - \left(\frac{[W_3 - W_2] \times 100)}{W_1}\right)$$

Protein Solubility as a Function of pH

Protein solubility of gelatin at different pH was determined according to Diniz and Martin [21] with slight modifications. 0.5g gelatin was dispersed in 50 mL distilled water at room temperature and the pH of the solution was adjusted to the desired values (3.0, 5.0, 7.0, 9.0 and 12.0) with either 0.5mol/L HCl or 0.5mol/L NaOH with continuous stirring for 45 minutes. At the end of this period, 25 mL aliquot was centrifuged at 3750 x g for 30 minutes. One millilitre aliquot of supernatant was analyzed for nitrogen content by the Kjedahl method and protein solubility was calculated according to the formula:

$$\text{Protein solubility (\%)} = \frac{\text{Protein content of supernatant} \times 100}{\text{Protein content of gelatin solution}}$$

Protein Solubility as a Function of Sodium Chloride Concentration

0.5g gelatin was separately dissolved in 50 mM of different concentrations of sodium chloride (0.5, 1.0, 1.5 and 2.0M) in 50 mL phosphate buffer (pH 7.5) [22]. The gelatin solution was centrifuged at 9000*g* for 15 minutes at 4°C. The total nitrogen content of the clear supernatant was determined by Kjeldahl method. Protein content was obtained by multiplying the nitrogen content by a factor of 5.4 according to Muyonga *et al.* [23]. Protein solubility as the percentage of total protein in the gelatin solution was calculated as follows:

$$\text{Protein solubility (\%)} = \frac{\text{Protein content of supernatant} \times 100}{\text{Protein content of gelatin solution}}$$

Emulsifying Capacity and Stability

Emulsion containing 1 g gelatin, 40 ml cold distilled water (4°C) and 40 ml sunflower oil was prepared according to Killekar *et al.,* [24] with slight modification. The emulsion was dispersed with a homogenizer (Wisemix HG-15D, Malaysia) at the speed of 9,600 rpm. Each homogenized sample was divided equally into 50 ml centrifuge tubes. One centrifuge tube was directly centrifuged at 4000 × g using a refrigerated bench top centrifuge (Universal 320R, Slovenia) for 10 minutes while the other was centrifuged under the same conditions after heating in a water bath at 80°C for 30 minutes and cooling to room temperature (25°C). The height of emulsified layer as percentage of total height of material in unheated tubes was used to calculate the emulsifying capacity and stability as follows:

$$\text{Emulsion capacity (\%)} = \frac{\text{Height of emulsion layer} \times 100}{\text{Height of whole layer}}$$

$$\text{Emulsion stability (\%)} = \frac{\text{Height of emulsion layer after heating} \times 100}{\text{Height of whole layer}}$$

Determination of Water Holding Capacity

Water holding capacity (WHC) was determined using the centrifugation method described by Diniz and Martin [21]. 1g gelatin was dissolved in 20 ml distilled water in centrifuge tubes and dispersed with a vortex mixer for 30 seconds. The dispersion was allowed to stand at room temperature for 6 hours, and then centrifuged at 2000 × g (KUBOTA, Japan) for 30 minutes. The dispersion was filtered with Whatman No. 1 filter paper and the volume recovered was recorded (supernatant). The WHC was calculated as the ml of water absorbed [difference between the volume of distilled water added to the gelatin sample (20 ml) and the volume of the supernatant recovered] per weight (gram) of gelatin sample.

$$\text{WHC (mL/g)} = \frac{\text{(volume of distilled water added – volume of supernatant recovered)}}{\text{Weight of gelatin}}$$

Determination of Fat-binding Capacities

0.5 g gelatin was placed in a centrifuge tube and weighed. 30 ml distilled water or 6 ml of soybean oil was added and held at room temperature for 1 hour. The gelatin solutions were vortexed for 5 seconds every 15 minutes followed by centrifugation at 450 × g for 20 minutes. The upper phase was removed and the centrifuge tube was drained for 30 minutes on a filter paper after tilting to 45° angle [25]. Fat binding capacities were calculated as the weight of the contents of the tube after draining divided by the weight of the gelatin.

Fat binding capacity:

$$= \frac{\text{(Wt of tube and content before draining)} - \text{(Wt of tube and content after draining)}}{\text{Weight of gelatin}} \times 100$$

Determination of Foaming Properties

1 g gelatin powder was added to 100 ml distilled water and homogenized for 1 minute at high speed, 10,600 rpm using a homogenizer (Wisemix HG-15D, Malaysia). The mixture was carefully and immediately transferred into a 250 ml measuring cylinder for volume measurement [26]. The foam capacity was calculated as the volume of mixture after homogenization

compared to the original. The whipped samples were allowed to stand for 30 minutes and the volume recorded. The foaming stability was calculated as the ratio of the foam capacity after 30 minutes divided by the original foam capacity.

Foam capacity (mL) = volume of mixture after homogenizing – volume of the original mixture

$$\text{Foam stability (\%)} = \frac{\text{foam capacity after 30 minutes} \times 100}{\text{Initial foam capacity}}$$

Results and Discussion

Solubility of Gelatin

Silver catfish skin gelatin was extracted at 50ºC at different time and the result showed that gelatin extracted for 12 hours had significantly ($p<0.05$) higher solubility compared to others (Table 1). Solubility also increased with increased in extraction time. According to Zayas [27], gelatin solubility depends on the molecular weight distribution and the content of polar and non-polar groups in amino acids. The increased in solubility of silver catfish gelatin with increased in extraction time could be due to the prolonged hot water exposure during extraction. Extraction in hot water breaks down the triple helical structure producing smaller and soluble gelatin molecules [10, 28].

Table 1: Solubility (%) of silver catfish skin gelatins extracted at 6, 8, 10 and 12 hours

	6 hours	8 hours	10 hours	12 hours
Solubility (%)	25.37 ± 0.97^{c}	35.22 ± 0.24^{b}	37.42 ± 0.35^{b}	45.10 ± 1.52^{a}

Data represent means of triplicate determinations ± standard deviation
Means within a row with different lowercases are significantly different ($p<0.05$)

Protein Solubility as a Function of pH

Solubility of protein is one of the most important characteristics because it influences other functional properties. Proteins with good solubility are required in many functional applications especially in foam, gel and

emulsion because it provides homogenous dispersal of the molecules in colloidal systems and enhanced the interfacial properties [27]. Solubility (%) of silver catfish skin gelatin as a function of pH is shown in Table 2. At pH 3, protein solubility were significantly ($p<0.005$) higher than others at all extraction time studied. The gelatin had low solubility at pH 7. This was in close agreement with [29] who obtained a maximum solubility of 95.8% at pH 2.4 for bigeye snapper gelatin while minimum solubility of 64.1% was observed at pH 6.4. Poppe [30] stated that gelatin is an amphoteric protein with isoelectric point between 5 and 9 depending on the raw material and method of manufacturing. The amphoteric character of gelatin is due to the functional groups of the amino acids and the terminal amino and carboxyl groups created during hydrolysis [31]. In strongly acidic solution, the gelatin is positively charged and migrates as a cation in electric field while protein tends to carry charges at pH values below and above the isoelectric point, thus enhancing hydration [31].

Table 2: Protein solubility (%) as a function of pH of silver catfish skin gelatins extracted at 6, 8, 10 and 12 hours

	Extraction time (hours)			
pH	6	8	10	12
3	80.67 ± 0.92^{bA}	74.30 ± 0.67^{cA}	87.21 ± 0.83^{aA}	87.11 ± 0.32^{aA}
5	45.99 ± 0.11^{bB}	67.65 ± 2.62^{aB}	69.72 ± 0.43^{aB}	69.57 ± 0.16^{aB}
7	6.64 ± 1.11^{cE}	30.96 ± 0.10^{bE}	51.51 ± 0.77^{aE}	51.58 ± 0.31^{aE}
9	26.03 ± 0.04^{cD}	46.45 ± 0.26^{bD}	58.10 ± 0.81^{aD}	59.02 ± 0.95^{aD}
12	41.22 ± 1.04^{dC}	55.14 ± 0.90^{cC}	62.13 ± 0.43^{bC}	64.05 ± 0.15^{aC}

Data represent means of triplicate determinations ± standard deviation
Means within a row with different lowercases are significantly different ($p<0.05$)
Means within a column with different uppercases are significantly different ($p<0.05$)

Protein Solubility as a Function of Sodium Chloride

Protein solubility is influenced by the number of polar and non polar groups and their arrangements along the molecules [32]. Protein solubility (%) as a function of sodium chloride of silver catfish skin gelatins are shown in Table 3. Protein solubility decreased with increased in NaCl concentration where maximum solubility was observed in 0.5 M NaCl and minimum solubility in

2.0 M NaCl. According to Damodaran and Kinsella [33], solubility increase with increases in salt concentration up to a certain level (salting 'in') and with further increase in salt concentration, the solubility decreases (salting 'out'). Protein solubility is also influenced by the extraction time. The longer the extraction time, the higher is the protein solubility (Table 3). During the extraction, acid treatment causes the skin to swell and non-collagenous protein leached out [34]. With prolonged heating time, the rate of collagen breakdown is enhanced thus more gelatinous protein is formed.

Table 3: Protein solubility (%) as a function of sodium chloride concentrations of silver catfish skin gelatins extracted at 6, 8, 10 and 12 hours

	Extraction time (hours)			
NaCl (M)	6	8	10	12
0.5	69.13 ± 0.63^{dA}	74.89 ± 0.25^{cA}	79.00 ± 0.87^{bA}	92.29 ± 0.15^{aA}
1.0	57.50 ± 0.30^{bB}	50.06 ± 0.13^{dB}	53.97 ± 0.78^{cB}	71.01 ± 0.93^{aB}
1.5	46.03 ± 0.23^{bC}	27.80 ± 0.12^{cC}	47.31 ± 1.99^{bC}	61.35 ± 1.00^{aC}
2.0	22.99 ± 0.13^{bcD}	22.15 ± 0.05^{cD}	23.56 ± 1.03^{bD}	54.76 ± 0.38^{aD}

Data represent means of triplicate determinations ± standard deviation
Means within a row with different lowercases are significantly different ($p < 0.05$)
Means within a column with different uppercases are significantly different ($p < 0.05$)

Emulsifying Capacity and Stability

Gelatin is used as emulsifying, foaming and wetting agent in food, technical, pharmaceutical and medical application due to its surface active properties [25]. Emulsifying capacity and stability of silver catfish skin gelatin increased with increased in extraction time (Table 4). Emulsifying capacity was higher at 12 hours extraction time. Borderias *et al.* [35] suggested that higher emulsifying capacity may be due to higher degree of unfolding of polypeptides. According to Ahmad and Benjakul [36] short peptide chains with more hydrophilic characteristics were preferably localized in aqueous phase. As a result, the lower portion of gelatin migrated to oil-water interface. Jellouli *et al.* [25] who observed that emulsion containing gelatin from grey triggerfish skin was more stable than that of the bovine gelatin stated that larger and longer peptides could stabilise the protein film at the interface more effectively.

Emulsion stability is the capacity of emulsion droplets to remain dispersed without separation by creaming, coalescing and flocculation [37]. In this study, silver catfish skin gelatin extracted for 12 hours present a good emulsifying characteristics compared to other samples. The protein in gelatin contains flexible polypeptide chains so that they are able to orient and unfold at the interface making them an effective emulsifier [38].

Water Holding Capacity

Water holding capacity refers to the ability of protein to imbibe water and retain it against a gravitational force within protein matrix [26]. Table 4 showed that water holding capacity of silver catfish skin gelatin increased with increased in extraction time. Water holding capacity was highest at 12 hours extraction time. Intrinsic factors such as amino acids composition, protein conformation, surface hydrophobicity or polarity and the amount of hydrophilic amino acids affect water binding capacity of food protein [39].

Fat Binding Capacity

Fat binding capacity is one of the functional properties that are closely related to the texture by interaction between components such as water and oil [22]. These functional properties depend on the degree of exposure of hydrophobic residues [25]. Longer extraction time produced gelatin characterized by higher fat binding capacities whereby gelatin extracted for 12 hours showed higher fat binding capacity than others (Table 4). According to McClements and Demetriades, [40], hydrophobic residues had a high affinity to non polar group and this interaction is highly dependent on temperature. A prolonged exposure to hot temperature (50ºC) during the extraction process could have probably exposed more hydrophobic residues within silver catfish skin gelatin thereby increasing its surface hydrophobicity. As a result, the gelatin produced was able to attract more non polar group and increasing its fat binding capacity.

Table 4: Functional properties of silver catfish skin gelatins extracted at 6, 8, 10 and 12 hours

Functional properties	Extraction time (hours)			
	6	8	10	12
Emulsifying capacity (%)	47.77 ± 0.29^{c}	50.20 ± 0.35^{ab}	51.33 ± 3.66^{ab}	52.63 ± 2.65^{a}
Emulsifying stability (%)	27.57 ± 3.55^{c}	30.23 ± 1.16^{c}	38.20 ± 0.20^{b}	47.83 ± 1.91^{a}
Water holding capacity (mL/g)	17.47 ± 1.54^{c}	22.21 ± 2.58^{bc}	26.96 ± 2.98^{ab}	31.78 ± 2.18^{a}
Fat binding capacity (%)	26.44 ± 3.59^{c}	27.37 ± 3.97^{bc}	31.93 ± 0.83^{b}	54.76 ± 0.76^{a}
Foaming capacity (mL)	12.67 ± 0.76^{c}	15.93 ± 1.83^{b}	39.50 ± 0.87^{a}	41.47 ± 2.15^{a}
Foaming stability (%)	13.90 ± 1.23^{d}	30.33 ± 0.83^{c}	46.87 ± 4.88^{b}	56.42 ± 4.53^{a}

Data represent means of triplicate determinations ± standard deviation
Means within a row with different lowercases are significantly different ($p<0.05$)

Foaming Properties

Table 4 showed that foam capacity and foam stability of silver catfish skin gelatin increased with increased in extraction time. Foaming stability of silver catfish skin gelatin extracted at 12 hours was significantly ($p<0.05$) higher than other samples. Foaming stability of silver catfish gelatin increased with increased in extraction time. According to Jellouli *et al.* [25], high content of hydrophobic amino acid such as valine, isoleucine, leucine, proline, methionine, phenylalanine and tyrosine had an influence on foam capacity. Koli *et al.* [26] stated that foam formation is generally controlled by the rearrangement, penetration and transportation of protein molecules at the interface. To express good foaming properties, protein

must be capable of migrating rapidly to the air-water interface, unfolding and rearranging at the interface [41]. Foam with higher concentration of protein was denser and more stable because of an increase in the thickness of interfacial films [27].

Conclusion

Gelatin from silver catfish skin was successfully extracted at 50ºC for 6, 8, 10 and 12 hours extraction times. Silver catfish gelatin was more effectively extracted at longer extraction time resulting in gelatin characterized by higher solubility, emulsifying properties, water holding capacity, fat binding capacities and foaming properties. Based on the results obtained, 12 hours extraction time is sufficient to produce gelatin from silver catfish skin with good functional properties and has the potential to be used as food additive.

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